REMARKS

Claims 36-68 are currently pending. Claims 37-59 have been cancelled, Claims 60-64 and 67 have been amended, and Claims 69-92 have been added. All other pending claims remain unchanged. The Applicants respectfully submit that no new matter is added through the proposed amendment to the claims.

The deletion of any claims and any claimed subject matter is being made solely to expedite allowance of the subject matter now claimed. The Applicants submit their amendments without prejudice to the subsequent prosecution of claims to some or all of the subject matter affected by any amendments made therein. The Applicants explicitly reserve the right to pursue the subject matter of any of the cancelled claims, or some or all of the subject matter affected by any amendments, in one or more Divisional or Continuation Applications.

1. Amendments to the Claims

Claim 60, as amended, introduces the language of now cancelled Claim 36. The language "whereby the linear peptide has a terminal glycine residue" and "through the terminal glycine residue" finds specific support, for example, in paragraph [0011] of the specification as filed. The language "combining both cell-targeting and therapeutic/diagnostic functions" and "wherein one face is grafted with at least one molecule of therapeutic or diagnostic interest, and the other is grafted with at least one recognition molecule of interest" finds specific support throughout the Specification as filed, for example, in Examples 1 and 2. Claims 61 and 62 have been amended to incorporate the language helpfully suggested by the Examiner. Claims 63 and 64 have been amended to be in conformance with Claim 60. In addition, Claim 64 has been amended to correct a typographical error. The subject matter of now cancelled Claims 36-59 has been reintroduced as newly added Claims 69-92.

No new matter is introduced with these amendments.

The Applicants respectfully request rejoinder of method Claims 67-92. The Applicants understand that method Claims 67-92 will be withdrawn from consideration until a product claim is found allowable, at which time method claims commensurate in scope with the allowed product claims will be rejoined in accordance with the provisions of MPEP 821.04.

2. **Objection to Claims 61 and 62**

The rejection objects to the use of "small letter" to designate D-amino acid residues in the recited peptide sequences. The objection is now moot in view of the amendments to claims 61 and 62. Accordingly, the Applicants respectfully request withdrawal of the stated objection.

3. **Rejection of Claims 60-62 and 66 under 35 U.S.C. § 103(a) over Scheibler *et al.* (Angew. Chem. Int. Ed. (1999) Vol. 38, pp. 696-699; hereinafter referred to as "Scheibler") in view of Kantlehner *et al.* (Angew. Chem. Int. Ed. (1999) Vol. 38, pp. 560-562; hereinafter referred to as "Kantlehner"), in further view of Rajopadhye *et al.* (WO 99/58162; hereinafter referred to as "Rajopadhye")**

Claims 60-62 and 66 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Scheibler in view of Kantlehner and in further view of Rajopadhye. The rejection states that Scheibler teaches a homodetic cyclopeptide tethered to a gold surface and functionalized with an antigenic $(NANP)_3$ derivative. However, the Applicants note with appreciation the Examiner's acknowledgement that Scheibler does not disclose SEQ ID NO:1 on one face of the cyclopeptide and a detection agent on the other.

The rejection turns to Kantlehner for its disclosure of the grafting of cyclopeptide c(RGDfK) onto a graft material (PMMA) to study the adhesion properties of the cyclopeptide to the integrin $\alpha v\beta 3$ receptor.

The rejection states that Rajopadhye teaches $\alpha v\beta 3$ binding pharmaceuticals attached to a therapeutic detecting agent through a linker.

The rejection then concludes that it would have been *prima facie* obvious to the skilled artisan to modify the homodetic cyclopeptide of Scheibler with the integrin $\alpha v\beta 3$ binding cyclopeptide of Kantlehner and the radiopharmaceutical of Rajopadhye.

The Applicants respectfully submit that the combination of cited references cannot support a *prima facie* case of obviousness for the reasons stated below.

The legal standard for establishing a *prima facie* case of obviousness requires that three basic criteria be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one skilled in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success in the

modification or in the combination; and (3) the prior art reference must teach all the claim limitations. All three requirements must be met to establish *prima facie* obviousness. In addition, the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure (MPEP 706.02(j)).

The combination of references fails *at least one* of the above three criteria, and therefore cannot be relied on to establish *prima facie* obviousness.

a. There is no motivation in the combination

The Applicants respectfully submit that none of the cited references suggest cyclic peptides so functionalized that they combine a cell-targeting moiety on one face of the cyclopeptide *and* a therapeutic/diagnostic moiety on the other face. In fact, Scheibler teaches cyclopeptides covalently bound to a solid support (*i.e.,* gold surface), leaving only one face of the cyclopeptide available for functionalization with a putative therapeutic/diagnostic molecule of interest, or a recognition molecule. It is thus clear, from reading Scheibler, that cyclopeptides whose faces are differentially functionalized to impart the cyclopeptide with (i) the ability to target cells, and (ii) a therapeutic/diagnostic property were not contemplated.

Kantlehner teaches a cyclic pentapeptide (cyclo(RGDfX)), which is selective for the $\alpha_v\beta3$ and $\alpha_v\beta5$ integrin receptors, covalently bound to a PMMA implant surface through a spacer. Only one face of Kantlehner's cyclopeptide is functionalized to permit anchoring on the implant surface. The underlying premise of Kantlehner's article is the preparation and use of surfaces covered with cell adhesion cyclopeptides "RDG", to be used as implants. Kantlehner did not and could not contemplate a cyclopeptide grafted on both faces, because doing so would destroy the intended purpose of the "RDG" cyclopeptides: their ability to bind the $\alpha_v\beta3$ and $\alpha_v\beta5$ integrin receptors.

The Applicants acknowledge that Rajopadhye teaches compounds having a targeting moiety covalently linked to a therapeutically or diagnostically useful radionuclide through an aliphatic or heteroaliphatic (*i.e.,* non-peptidic) linker. However, there is no suggestion nor teaching in any of the cited references to substitute the non-peptidic linker of Rajopadhye with a homodetic cyclopeptide, much less to differentially functionalize each face of the cyclopeptide with a recognition molecule on the one hand and a molecule of therapeutic or diagnostic interest on the other.

Thus, the Applicants respectfully submit that the combination of cited references cannot rise

to the level of *prima facie* obviousness because there is no motivation or suggestion in any of the cited references, or in the general knowledge in the art, to modify the teachings of Scheibler, Kantlehner and/or Rajopadhye. Withdrawal of the §103 rejection is earnestly requested.

b. There is no reasonable expectation of success in the combination

The Applicants respectfully submit that the rejection has not established that there exists the required reasonable expectation of success within the combination of the cited references. The teachings, suggestions, and expectation of success must come from the prior art, not the Applicants' disclosure. See *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991). The Applicants respectfully submit that the cited references provide no reasonable expectation of success.

The Applicants turn to Peri *et al.* Tetrahedron, 1998, 54, 12269-12278 ("Peri"), which describes a method for the stereoselective coupling of unprotected oligosaccharides with a substrate containing a N,O-disubstituted hydroxylamine group (See Abstract). [A copy of the Peri reference is provided herewith for the convenience of the Examiner.]

Peri teaches β-N-glycosylation of a model peptide (See paragraph 2 page 12271), which is the linear peptide Lys-Ala-Lys (See figure 3 page 12271). The reaction leads to the formation of a derivative where the saccharide and the substrate (*e.g.*, peptide) are linked through a disubstituted hydroxylamine bond (See Page 12270, §2, Fig. 1). Peri does not contemplate homodetic cyclopeptides, much less cyclopeptides grafted via an oxime bond.

The Applicants invite the Examiner'attention to Page 12269, Lines 15-17 of the Peri reference, which reads:

> However, the presence of the E/Z oxime isomers or equilibrium between cyclic and linear oxime-sugar forms has lead to conformational heterogeneity of the glycoconjugate that may significantly hamper the general scope and potentiality of these convenient approaches.

Thus, Peri teaches that the use of an oxime bond in preparing glycoconjugates (e.g., glyopeptides) may be undesirable due to the existence of the oxime functional group as two isomeric forms (E/Z). Accordingly, the skilled artisan, armed with the knowledge of Peri, would not be motivated to combine the teachings of Scheibler, Kantlehner and Rajopadhye to prepare grafted homodetic cyclopeptides wherein at least one molecule of interest is grafted onto the upper or lower face of the framework via an oxime bond, because Peri specifically teaches that the use of an oxime bond to

prepare such bioconjugates is not desirable. The general knowledge available in the art would thus lead the skilled practitioner to conclude that there existed no reasonable expectation of success in the combination of cited references. Therefore, the combination of Scheibler, Kantlehner and Rajopadhye cannot render the solicited claims obvious.

c. The references do not teach all the claim limitations

The Applicants respectfully submit that neither the Scheibler, Kantlehner nor Rajopadhye reference teaches or suggests a grafted homodetic cyclopeptide combining both cell-targeting and therapeutic/diagnostic functions, whereby at least one molecule of therapeutic or diagnostic interest is grafted on one face of the cyclopeptide framework, and at least one recognition molecule of interest is grafted on the other face of the cyclopeptide framework, as recited in Claim 60. A necessary criterion for establishing *prima facie* obviousness is that the prior art reference or references must teach or suggest all claim limitations. See Manual of Patent Examining Procedure, section 2143.03:

> To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re* Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). 'All words in a claim must be considered in judging the patentability of that claim against the prior art.' *In re* Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970).

Scheibler, Kantlehner and Rajopadhye fail to teach or suggest a grafted homodetic cyclopeptide combining both cell-targeting and therapeutic/diagnostic functions, whereby at least one molecule of therapeutic or diagnostic interest is grafted on one face of the cyclopeptide framework, and at least one recognition molecule of interest is grafted on the other face of the cyclopeptide framework. In fact, the Applicants respectfully submit that Scheibler and Kantlehner *teach away* from this very proposition. As seen above, Scheibler and Kantlehner teach cyclopeptides covalently bound to a solid support (*i.e.*, gold surface in Scheibler and a PMMA implant surface in Kantlehner). In other words, one face of the cyclopeptide in Scheibler and Kantlehner is obstructed by the solid support, leaving only one face of the cyclopeptide available for functionalization with a putative therapeutic/diagnostic molecule of interest, or a recognition molecule. Furthermore, functionalizing the face that remains available in Kantlehner's "RDG" cyclopeptide would destroy Kantlehner's cyclopeptide intended function (*i.e.*, its ability to bind the $\alpha_v\beta3$ and $\alpha_v\beta5$ integrin receptors). Accordingly, Scheibler and Kantlehner teach away from the claimed homodetic

cyclopeptides wherein at least one molecule of therapeutic or diagnostic interest is grafted on one face of the cyclopeptide framework, and at least one recognition molecule of interest is grafted on the other face of the cyclopeptide framework. Thus, the cited references do not teach or suggest all the claim limitations.

Accordingly, there is nothing in the disclosure of Scheibler, Kantlehner and Rajopadhye that would provide one of ordinary skill in the art any motivation to make and use the claimed grafted cyclopeptides. As such, Claim 60, as amended, and claims that depend from it, are not rendered obvious by the disclosure of Scheibler, Kantlehner and Rajopadhye.

Any one of the remarks a-c above *alone* overcomes the rejection under 35 U.S.C. § 103(a). Thus, the combination of cited references does not render obvious the subject matter recited in the solicited claims. It follows that the cited references do not support *prima facie* obviousness of Claims 60-62 and 66.

In addition, the following remarks further support Applicant's position that the combination of cited references fails to establish *prima facie* obviousness.

d. Evidence of unexpected results thwarts the presumption of obviousness

The claims are directed to grafted homodetic cyclopeptides obtained by a process comprising intramolecularly cyclizing a linear peptide having a terminal glycine residue.

Scheibler teaches preparation and use of a cyclodecapeptide on a gold surface. Scheibler prepared the cyclodecapeptide according to the method described in Dumy *et al.*, and reported in Tetrahedron Letters, 1995, Vol 36, pp. 1255-1258 (See reference 14 cited on page 696 of the Scheibler reference). The above-referenced Tet. Lett. article describes the preparation of cyclopeptides from linear peptides whereby cyclization is effected through a terminal *lysine* residue – not a glycine residue. The synthetic approach disclosed in the Tet. Lett. article presents a main drawback: the possibility of side chain epimerization at the lysine residue during cyclization. This side reaction may in turn lead to the formation of a mixture containing the desired cyclopeptide and an undesired side product (the lysine residue epimer). This method is therefore not recommended for, nor amenable to, preparation of cyclopeptides for therapeutic/diagnostic applications such as those claimed by the Applicants.

One of ordinary skill in the art, reading the Tet. Lett. article, would understand that the cyclization reaction via lysine residue described in the Tet. Lett. reference was used because it was

thought to be the easiest to achieve at the time. In particular, given the structures of the cyclopeptides described in the Tet. Lett. article, the skilled practitioner would not have been motivated to prepare the cyclopeptides by effecting cyclization via a terminal glycine residue. Specifically, given the presence of the two β-turns due to the Pro-Gly sequence in the resulting cyclopeptide disclosed in the Tet. Lett. article, the skilled artisan would have considered a cyclization reaction via a terminal proline or glycine residue to be very difficult, if not impossible, to achieve. Notwithstanding a lack of motivation in the art to utilize a terminal glycine residue to effect the cyclization step, the Applicants surprisingly demonstrated that cyclization through a terminal *glycine* residue was not only feasible, but that it also proceeded with comparable efficiency. In addition, it solved the risk of epimerization that can occur when the cyclization reaction is effected through an amino acid residue other than glycine. This result was unexpected and unsuggested in the art.

Scheibler, Kantlehner and Rajopadhye fail to suggest reasons for selecting a terminal glycine residue as a result effective variable. Indeed, none of the cited references teaches or suggests a method for preparing the cyclopeptides disclosed in the cited art other than the method described in the aforementioned Tet. Lett. article. The rejection appears to rely solely on the structural similarity of the art and Applicants' grafted cyclopeptides, and the expectation of obtaining similar properties for structurally similar compounds. As the CCPA established *In re Papesch,* structural similarity is not sufficient to support a finding of obviousness in the face of unexpected or superior properties among similar compounds. *In re Papesch*, 315 F.2d at 382, 137 USPQ at 44. Papesch claimed certain trialkyl copounds, in which the alkyl groups contained "more than one or less than five carbon atoms." Id. The USPTO rejected Papesch's claims based on prior art disclosing a trimethyl group, reasoning that the prior art compound's structural similarity made the claimed compounds obvious. In response, Papesch filed a declaration reporting the results of testing that showed a triethyl compound of the invention had anti-inflammatory activity while the trimethyl did not. he Board sustained the examiner's rejection, giving no weight to the evidence submitted in the declaration. In finding the declaration's evidentiary showing persuasive of the claimed invention's patentability, the CCPA reversed the Board and stated:

> [W]e think that [the Board's rejection] rests on one fundamental error of law, namely, the failure to take into consideration the biological or pharmaceutical property of the compounds as anti-inflammatory agents on the ground that to chemists the structure of the compounds would be so obvious as to be beyond doubt, and that a showing of

> such property is to be used only to resolve doubt.
> From the standpoint of patent law, a compound and all of its properties are inseparable; they are one and the same thing... And the patentability of the thing does not depend on the similarity of its formula to that of another compound but of the similarity of the former compound to the latter. There is no basis in law for ignoring any property in making such a comparison. An assumed similarity based on a comparison of formulae must give way to evidence that the assumption is erroneous.

Here, the Applicants provided evidence in the form of the Examples described in the Application as filed (under oath) that cyclization of the linear peptide via a terminal glycine residue imparts greater purity to the claimed grafted cyclopeptides (*i.e.,* eliminates the risk of epimerization in the cyclization step). The recognition by the Applicants, unsuggested in the cited art, that the use of a terminal glycine residue to effect the cyclization step leads to grafted cyclopeptide of superior quality is the touchstone of unobviousness. Thus, the combination of cited art cannot render the pending claims obvious. It is therefore respectfully requested that the rejection be reconsidered and withdrawn.

e. The cited art does not disclose any method for making structurally similar products

It is well established that, where no known or obvious method exists for making a compound, merely naming or suggesting a compound will not constitute a description of the compound and will not place it in possession of the public. See *In re Hoeksama,* 399 F.2d at 273, 158 USPQ at 600. The court *In re Hoeksama* held:

> [U]pon careful consideration it is our view that if the prior art of record fails to disclose or render obvious a method for making a claimed compound, at the time the invention was made, it may not legally be concluded that the compound itself is in possession of the public. In this context, we say that the absence of a known or obvious process for making the claimed compounds overcomes a presumption that the compounds are obvious, based on close relationships between their structures and those of prior art compounds.

In this case, none of the cited references contemplate cyclopeptides grafted on both faces with molecules of interest for (i) recognition purposes and (ii) therapeutic/diagnostic purposes. The Applicants acknowledge that Scheibler teaches a cyclopeptide "functionalized" on both faces. However, the "functionalization" of one of the faces of Scheibler's cyclopeptide is reduced to *covalent anchoring to a gold surface,* which has little to do with the Applicants' functionalization

with a recognition molecule or a molecule of therapeutic/diagnostic interest. Thus, Scheibler does not teach synthetic approaches that would allow the *de novo* synthesis of grafted homodetic cyclopeptides combining both cell-targeting and therapeutic/diagnostic functions, wherein one face is grafted with at least one molecule of therapeutic or diagnostic interest, and the other is grafted with at least one recognition molecule of interest, such as those recited in the Applicants' claims. Kantlehner and Rajopadhye do not teach nor suggest cyclopeptides grafted on both faces, much less methods for making them. Thus, the combination of cited references cannot render obvious the grafted homodetic cyclopeptides recited in the present claims. It follows that the cited references cannot support *prima facie* obviousness of Claim 60.

Since independent Claim 60, as currently amended, is not rendered obvious in light of Scheibler in view of Kantlehner and in further view of Rajopadhye, and since all pending claims depend ultimately from Claim 60, it follows that all the claims, as amended, are patentable over the cited references.

In light of the above remarks, the combination of Scheibler, Kantlehner and Rajopadhye cannot render the instant claims obvious. The Applicants respectfully request that the outstanding rejection under 35 U.S.C. §103 be withdrawn.

CONCLUSIONS

In light of the foregoing Remarks, the Applicants respectfully submit that the present Application is in condition for allowance. A Notice to that effect is respectfully requested. If the Examiner believes that a telephone interview would be of assistance in advancing the prosecution of this application, the Examiner is invited to telephone the undersigned.

Respectfully submitted,

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TETRAHEDRON

Chemo- and Stereoselective Glycosylation of Hydroxylamino Derivatives : A Versatile Approach to Glycoconjugates

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Abstract: A general method for the stereoselective coupling of unprotected oligosaccharides with any substrate containing a N,O-disubstituted hydroxylamine group is described. The cyclic nature of the oligosaccharide reducing unit is preserved and the substrate glycosylated with high diastereoselectivity to sugar through an amino (N[OR_2]-) or an aminoxy (N[R_1]-O-) linkage. Due to the uniquely high chemical reactivity and specificity of disubstituted hydroxylamine toward the sugar reducing end, neither protecting groups nor activation methods are required to perform the reaction in aqueous solution. The characteristic features and the scope of this new type of glycosylation reaction are exemplified for the chemoselective synthesis of model glycopeptides.

Keywords: Glycopeptides; Glycosylation; Stereocontrol.

INTRODUCTION

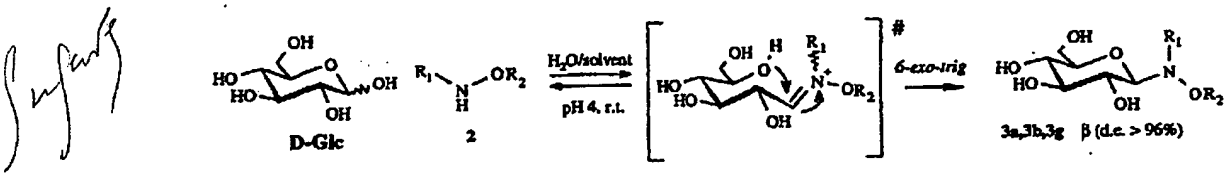
The critical role of specific oligosaccharide structures in the biological function of many glycoproteins is now well appreciated[1]. The importance of protein-bound oligosaccharides in cell-cell recognition events and in modulating protein folding and stability has been highlighted in a number of recent landmark studies, inspiring the development of elegant chemical[2] and enzymatic[3] approaches for the construction of glycoproteins with defined, homogeneous glycoforms. However, the regiospecific chemical approach to glycoconjugates remains a challenging task due to the requirement for extensive protecting group manipulations and the chemical sensitivity of glycosidic linkages. Convergent methods exploiting available sources of carbohydrates appear to give flexible and rapid entry to the synthesis and functional screening of carbohydrates-based-antigens or conjugates. In this context, methods employing the [redacted] have been introduced recently for rapid access to neoglycopeptides. Chemoselective ligation methodologies[4] are based on the introduction of mutually and uniquely reactive moieties (e.g. a hydroxylamino group and an aldehyde group) on to fragments that makes coupling of these fragments possible in aqueous solution without protecting groups or activation procedures. Oxime-bound carbohydrate molecules have been readily obtained from ligation of unprotected oligosaccharides to free-hydroxylamino-peptide fragments[5,6] and α-O-glycosylated peptide-aldehydes to hydroxylamino-galactose derivatives[7]. However, the presence of the E/Z oxime isomers or equilibrium between cyclic and linear oxime-sugar forms has led to conformational heterogeneity of the glycoconjugate that may significantly hamper the general scope and potentiality of these convenient approaches.

0040-4020/98/$ - see front matter
PII: S0040-4020(98)00763-7

We study here the glycosylation of *N,O*-substituted hydroxylamines with reducing sugars (Scheme 1). We report that, in contrast to oxime formation[5] or reductive amination[8] methods, the cyclic nature of the saccharide reducing unit is completely preserved. We also demonstrate that the reaction proceeds chemoselectively and with high anomeric stereoselection without requirement of activation at the anomeric center and protection of functional groups. We illustrate some possible applications conjugating a model tripeptide with natural mono- di- and trisaccharides.

RESULTS AND DISCUSSION

In order to assess the potential of this approach, alkyl *N,O*-substituted hydroxylamines 2 were reacted with available carbohydrate molecules 1a-g. We were pleased to find that the reaction effectively provides the expected glycosylated compounds 3a-g[9] in good and reproducible yields (Table 1). The reaction proceeded under mild conditions in aqueous buffer (pH 4) or in polar organic solvents (generally a mixture of acetic acid and DMF) and was generally complete within 20 hours for all carbohydrates but mannose and lactose.



Scheme 1. Principle of the chemoselective glycosylation of *N,O*-substituted hydroxylamine 2 with D-Glucose.

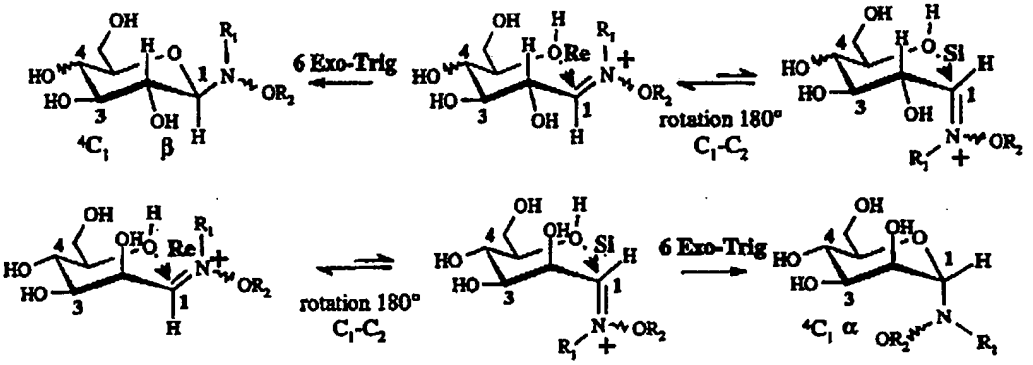
Compounds 3a-g were obtained exclusively in the pyranose form with a typical $^4C_1$ conformation as ascertained by NMR investigations[10,11] in solution except for galactose and mannose derivatives 3d and 3f, where 20% and 13% of the furanose form was also detected respectively[12,13].

Table 1. Yields and Reaction Times of Glycosylation of *N,O*-substituted Hydroxylamines.

Product	Sugar 1a-g	R_1	R_2	Times (h)	Yield (%)
3a	L-Glc	Et	Bn	20	90
3b	D-Glc	Me	Me	20	92
3c	D-GlcNAc	Me	Me	20	40
3d	D-Gal	Et	Bn	20	80
3e	Lactose	Me	Me	60	95
3f	D-Man	Me	Me	144	60
3g	D-Glc	Octyl	Me	20	30

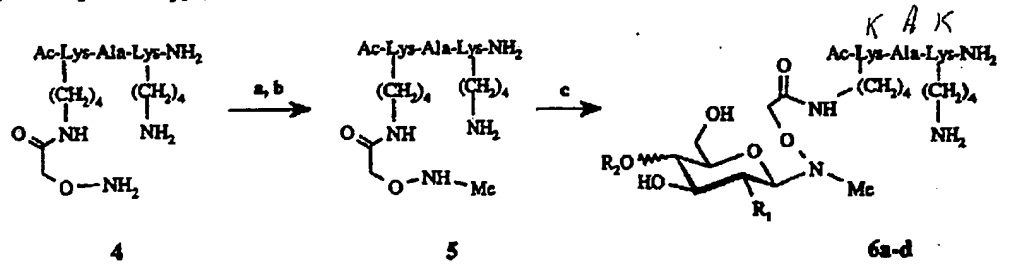
Noteworthy is the analysis of $^3J_{H1\text{-}H2}$ and $^1J_{^{13}C1\text{-}H1}$ coupling constant values which indicates that the reaction proceeds with high diastereoselectivity (d.e. > 96%)[10,11]. Only the β-pyranose forms were observed for the glucose and galactose derivatives (3a-e and 3g), whereas for the mannose derivative 3f the α anomeric configuration was preferred. These high diastereoselectivities are indicative of a thermodynamic control of the

intermediate during the ring closure reaction. The presence of an oxy-imminium intermediate (Scheme 1) is supported by *in situ* $NaBH_3CN$ quenching of the reaction which afforded the corresponding open chain hydroxylamine derivative. The β or α pyranose forms obtained here can be rationalised by a preferential 6-exo-trig ring closure with OH-5 attacking the Re or Si oxy-iminium face respectively (Scheme 2) while the furanoside forms originate from 5-exo-trig ring closure with OH-4.



Scheme 2. 6-exo-trig ring closure of the oxy-iminium intermediate with OH-5 attacking preferentially the Re or Si face to afford diastereoselectively β-galacto or β-gluco (top) and α-manno (bottom) derivatives 3a-g.

With these results in hand, we exploited the metho[redacted] peptides (Scheme 3). N[Me]-O-peptide 5 was obtained simply by reduction of the formaldehyde-oxime of the corresponding primary aminooxy peptide 4 (Scheme 3, steps a and b). As exemplified in Table 2 for D-glucose derivatives, the desired peptides 6a-d were obtained in moderate to good yields with the β-pyranose configuration as ascertained by NMR spectroscopy ($^3J_{H1-H2} > 8$ Hz).



4 5 6a-d

Scheme 3. Preparation and chemoselective glycosylation of aminooxy-peptide 5 with D-Glucose derivatives. a) CH_2O, Aqueous sodium acetate buffer, pH 4. b) $NaBH_3CN$, AcOH. c) Aqueous sodium acetate buffer, pH 4, 60°C, sugar.

Analysis of the reaction course by HPLC indicates that a single product was formed corresponding after NMR analysis to the expected glycoconjugates 6a-d. In particular, in the ROESY spectra the presence of a ROE cross peak between the H-1 of the sugar unit and the N-Me hydrogens of the hydroxylamino group is of diagnostic value for the regioselectivity toward the hydroxylamino moiety and thus rules out a possible parallel

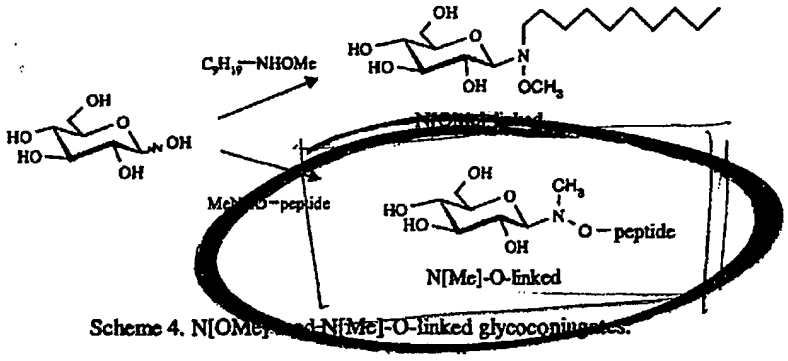
amino glycosylation with the free lysine amino group. This demonstrates the chemoselectivity of our hydroxylamino glycosylation even under long reaction times and harsh conditions (60°C). Consequently, neither protecting groups nor activation methods are required to perform the glycosylation step which represents an interesting alternative to existing chemical approaches to glycopeptides[2].

Table 2. Yields and Reaction Times of Glycosylation of Peptide 5.

Product	Sugar	Time (h)	Yield (%)
6a	D-Glc	48	62
6b	D-Lactose	144	57
6c	Maltotriose	144	35
6d	GalNAc	48	70

These reaction conditions and the very good stereoselectivity observed open up the possibility to glycosylate unprotected peptides directly on a solid support. This may then allow the use of an excess of sugar to improve the yields of glycosylation as well as the recycling of that unreacted material by filtration.

Although the products we obtained here do not have a natural aglycon linkage, the conformation of the first sugar unit which is thought to play a dominant role in biological activity[14,15] is preserved. Therefore glycoconjugates of this type may prove useful in glycobiology as mimic of natural occurring glycosylated molecules. Another advantage of using *N,O* substituted hydroxylamine is that the oligosaccharide chain can be conjugated either to R_2 through an aminooxy (N[R_1]-O-, 6a-d) linkage or to R_1 through an amino (N[OR_3]-, 3g) linkage depending on how the substrate is tethered to the hydroxylamine (Scheme 4). Compounds 3g is an illustrative example of a N[OR_2]- conjugation of a lipid moiety (octyl) to glucose which after reduction of the N-O bond (Zn / AcOH, 30 min 85%) provides an efficient synthetic entry to the corresponding N-Octyl-(β-D-glucopyranosyl)amine known for immunostimulation properties[16].



Scheme 4. N[OMe]- and N[Me]-O-linked glycoconjugates.

CONCLUSION

The results obtained herein demonstrate that the reaction of an *N,O*-substituted hydroxylamine with carbohydrates provides a rapid synthetic entry to stereoselectively glycosylated molecules such as peptides or lipids with naturally or chemically accessible oligosaccharides. The reaction makes use of oxime bond chemistry but without its usual inconveniences. This should provide a very efficient method for the rapid synthesis of more complex targets such as carbohydrate-based antigens for immunological studies.

EXPERIMENTAL SECTION

Materials and Methods. Reagents and solvents were purchased from Fluka (Buchs, CH) and used without further purification. All protected amino acids were purchased from Calbiochem-Novabiochem (Laufelfingen, CH). HPLC was performed on Waters equipment using column packed with Vydac Nucleosil 300 Å 5 μm C_{18} particles unless otherwise stated. The analytical column (250 x 4.6 mm) was operated at 1 mL/min and the preparative column (250 x 21 mm) at 18 mL/min, monitoring at 214 nm. Solvent A consisted of 0.09% TFA and solvent B of 0.09% TFA in 90% acetonitrile unless otherwise stated. Mass spectra were obtained by electron spray ionisation (ESI-MS) on a Finningan MAT SSQ 710C. NMR spectra were recorded in D_2O at 400 MHz (Bruker ARX spectrometer) or at 600MHz (Bruker AMX-2 spectrometer) at 300 K. 2D experiments were typically acquired using 2K x 512 matrices over a 2000 Hz sweep width in both dimensions. Quadrature detection in the indirect dimension was achieved by using the TPPI procedure[17]. Scalar connectivities were recovered from 2D double quantum filtration (DQF) COSY experiments[18]. Dipolar connectivities were obtained either through the conventional NOESY sequence[19] or the ROESY sequence[20] with mixing times from 150 to 200 ms. A randomisation of the mixing length (± 5%) was introduced in the NOESY experiments in order to minimise coherence transfer. The spin lock mixing interval of the ROESY sequence was applied by coherent CW irradiation at $\gamma B_2/2\pi$ = 1 KHz. Experimental data processing was performed using the Felix software package. The standard sinebell squared routine was employed for apodization with a shift range of 60-90° and zero filling in both dimensions before 2D transformations were applied to end up with square matrices of 2K x 2K real point data. Proton resonance assignments were made using (DQF) COSY experiments, HMQC was used to assign unambiguously the carbon resonances. J coupling constants were directly measured from high-resolution ^{1}H-1D spectra (±0.2 Hz).

N-Ethyl-O-benzylhydroxylamine (2a). A solution of *O*-benzylhydroxylamine hydrochloride (1g, 6.3 mmol), AcONa (1g) and acetaldehyde (12.6 mmol, 720 μl) in water/methanol (100 mL, 5:1 mixture), was stirred at room temperature for 10 min. The solvent was concentrated *in vacuum* and the residue was taken up in ethyl acetate (200 mL), washed with aqueous citric acid (200 mL, 10% w/v), dried over sodium sulphate and evaporated to dryness to afford the oxime as a colourless oil (0.87 g, 92%) which was used subsequently without further purification. A solution of the *O*-benzyl oxime of acetaldehyde (0.87 g, 5.8 mmol) in acetic acid (3mL) was treated with $NaCNBH_3$ (1.46 g, 4 equiv.) for 1 h at room temperature. The reaction mixture was concentrated, the residue taken up in diethyl ether (200 mL), washed with brine, dried on sodium sulphate, filtered, and concentrated. The product was purified by flash chromatography on silica-gel (25% ethyl acetate/hexane) and recovered as a colourless oil (0.7 g, 80% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.13 (t,

3H, CH_3, ^3J = 7.0 Hz), 3.01 (q, 2H, CH_2-N, ^3J = 7.0 Hz), 4.74 (s, 2H, CH_2-OPh), 5.39 (bs, 1H, NH), 7.26-7.41 (m, 5H, H_{arom}); ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 14.8 (CH_3), 48.8 (CH_2-N), 78.6 (CH_2-O), 130.2,130.7, 140.2 (C_{arom}); MS: m/z = 152 (M + H$^+$), 91; Anal. Calcd. for $C_9H_{13}NO$ (151.20): C 71.49, H 8.67, N 9.26. Found: C 71.51, H 8.71, N 9.34.

N-Ethyl-O-benzyl-N-(β-D-glucopyranosyl)hydroxylamine (3a). A solution of D-glucose (50 mg, 0.28 mmol) in 4 mL of DMF/AcOH 50% and *N*-Ethyl-*O*-benzylhydroxylamine (63 mg, 0.42 mmol), was stirred for 20 h at room temperature. After removal of the volatile, the product was purified by flash chromatography on silica gel (10% methanol/ethyl acetate) and recovered as a colourless oil which precipitated from diethyl ether as a white solid (78 mg, 90%). $[\alpha]^{25}_D$ = -4.6 (*c* = 0.4, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 1.12 (t, 3 H, $\underline{CH_3}CH_2N$, ^3J = 7.0 Hz), 2.91 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J =12.0 Hz, ^3J =7.0 Hz,), 3.11 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J = 12.0 Hz, ^3J = 7.0 Hz), 3.26 (m, 1H, H4), 3.27 (m, 1H, H5), 3.43 (dd, 1H, H3, $^3J_{2-3}$= $^3J_{3-4}$ = 9.0 Hz), 3.58 (dd, 1H, H2, $^3J_{2-3}$= $^3J_{1-2}$ = 9.0 Hz), 3.60 (dd, 1H, H6, $^2J_{6-6'}$ = 12.0 Hz, $^3J_{6-5}$ = 1.6 Hz), 3.76 (dd, 1H, H6', $^2J_{6-6'}$ = 12.0 Hz, $^3J_{6'-5}$ = 1.6 Hz), 4.12 (d, 1H, H1, $^3J_{1-2}$ = 9.0 Hz), 4.75 (2H, CH_2Ph), 7.3 (m, 5H, H_{arom});^{13}C NMR (400 MHz, D_2O): δ =13.9 ($\underline{C}H_3CH_2N$), 49.7 ($CH_3\underline{C}H_2N$), 63.0 (C6), 71.6 (C4), 72.2 (C2), 79.3 ($\underline{C}H_2$Ph), 79.5 (C3), 79.5 (C5), 93.9 (C1), 131.0, 131.1, 131.7, 138.0 (C_{arom}); MS (ESI): m/z = 314.3 (M + H$^+$). Anal. Calcd. for $C_{15}H_{23}NO_6$ (313.3): C 57.50, H 7.40, N 4.47. Found: C 57.39, H 7.28, N 4.32.

N,O-Dimethyl-N-(β-D-glucopyranosyl)hydroxylamine (3b). A solution of D-glucose (90 mg, 0.5 mmol) and *N,O*-dimethyl-hydroxylamine hydrochloride (195 mg, 2 mmol) in 5 mL of aqueous buffer (sodium acetate/acetic acid 0.1 M, pH 4), was stirred for 20 h at room temperature. The solvent was removed and flash chromatography (water/methanol/ethyl acetate = 1:24:75) afforded the pure product as a colourless oil (102 mg, 0.46 mmol, 92%). $[\alpha]^{25}_D$ = -9.5 (*c* = 1.2, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 2.66 (s, 3 H, CH_3N), 3.24-3.34 (m, 2H, H3 and H5), 3.38-3.47 (m, 2H, H2 and H4), 3.49 (s, 3H, CH_3O), 3.64 (dd, 1H, H6, $^3J_{5-6}$ = 5.4 Hz, $^2J_{6-6'}$ = 12.1 Hz), 3.83 (dd, 1H, H6', $^3J_{5-6}$ = 1.8 Hz, $^2J_{6-6'}$ = 12.1 Hz), 4.02 (d, 1H, H1, $^3J_{1-2}$ = 8.5 Hz);^{13}C NMR (400 MHz, D_2O): δ = 40.35 (CH_3N), 62.28 (CH_3O), 63.23 (C6), 71.82 (C3), 72.10 (C4), 79.42,(C2), 79.71 (C5), 95.19 (C1); MS: m/z = 224 (M+H$^+$), 192 (M-OMe); Anal. Calcd. for $C_9H_{17}NO_6$ (223.2): C 43.05, H 7.68, N 6.28; found. C 43.25, H 7.76, N 6.22.

N,O-Dimethyl-N-(2-aminoacetyl-2-deoxy-β-D-glucopyranosyl)hydroxylamine (3c). *N*-Acetyl-glucosylamine (100 mg, 0.45 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (176 mg, 1.8 mmol) were reacted using the same procedure described for 3b. The crude material was purified by chromatography (water/methanol/ethyl acetate = 1:24:75) affording, after precipitation from diethyl ether, 47 mg of 3c, (40%). $[\alpha]^{25}_D$ = +3.1 (*c* = 2.2, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 1.90 (s, 3H, CH_3CO), 2.58 (s, 3 H, CH_3N), 3.25-3.30 (m, 1H, H4), 3.30 (s, 3H, CH_3O), 3.30-3.45 (m, 1H, H3), 3.55-3.65 (m, 1H, H5), 3.65-3.80 (m, 2H, H6 and H6'), 3.84 (dd, 1H, H2, $^3J_{2-3}$ = $^3J_{1-2}$ = 9.8 Hz), 4.06 (d, 1H, H1);^{13}C NMR (400 MHz, D_2O): 17.8 ($\underline{C}H_3CO$), 35.4 (CH_3N), 56.5 (CH_3O), 58.0 (C5), 58.1 (C2), 66.6 (C6), 72.6 (C3), 74.4 (C4), 88.3 (C1), 171.5 ($CH_3\underline{C}O$); MS: m/z = 265 (M+H$^+$), 234 (M- OMe); $C_{10}H_{20}N_2O_6$ (264.3) calcd. C 45.44, H 7.63, N 10.60; found C 45.47, H 7.71, N 10.57.

N-ethyl-O-benzyl-N-(β-D-galactosyl)hydroxylamine. D-galactose (50 mg, 0.28 mmol) and *N*-ethyl-*O*-benzylhydroxylamine (63 mg, 0.42 mmol) were reacted using the same protocol as for 3a. The product was purified to afford 52 mg of a colourless oil (60%). The product consisted of a mixture of the β-pyranose (80%) and the β-furanose (20%) forms. MS (ESI): m/z = 314.3 (M + H$^+$); Anal. Calcd. for $C_{15}H_{23}NO_6$ (313.3): C

57.50, H 7.40, N 4.47; found: C 57.42, H 7.37, N 4.49. As it was not possible to separate the two forms by chromatographic methods the two compounds were characterised as a mixture: *N-Ethyl-O-benzyl-N-(β-D-galactopyranosyl)hydroxylamine (3d).* ^{1}H NMR (400 MHz, D_2O): δ = 1.09 (t, 3 H, $\underline{CH_3}CH_2N$, ^3J = 7.2 Hz), 2.91 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J=12.7 Hz, ^3J=7.2 Hz,), 3.07 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J = 12.7 Hz, ^3J = 7.2 Hz), 3.49-3.68 (m, 4H, H3-H5-H6-H6'), 3.73 (dd, 1H, H2, $^3J_{1-2}$= $^3J_{2-3}$= 9.1 Hz), 3.80 (d, 1H, H4, $^3J_{3-4}$ = 3.3 Hz), 4.08 (d, 1H, H1, $^3J_{1-2}$ = 9.1 Hz), 4.77 (2H, CH_2Ph), 7.3 (m, 5H, H_{arom}); ^{13}C NMR (400 MHz, D_2O): δ = 14.0 ($\underline{C}H_3CH_2N$), 48.9 ($CH_3\underline{C}H_2N$), 63.5 (C6), 69.7 (C2), 71.4 (C4), 76.5 (C3), 78.9 (C5), 79.1 ($\underline{C}H_2Ph$), 94.7 (C1), 131.0, 131.7, 138.0 (C_{arom}). *N-Ethyl-O-benzyl-N-(β-D-galactofuranosyl)hydroxylamine.* ^{1}H NMR (400 MHz, D_2O): δ =1.05-1.15 (m, 3 H, $\underline{CH_3}CH_2N$), 2.8–3.1 (m, 2H, $CH_3\underline{CH_2}N$), 3.5-3.8 (m, 4H, H4,H5, 2H6), 4.04 (dd, 1H, H3, $^3J_{2-3}$= $^3J_{3-4}$= 7.0 Hz), 4.24 (dd, 1H, H2), 4.47 (d, 1H, H1, $^3J_{1-2}$= 7.0 Hz), 4.7-4.8 (m, 2H, CH_2Ph).

N,O-Dimethyl-N-(β-D-lactosyl)hydroxylamine (3e). D-Lactose monohydrate (100 mg, 0.28 mmol) was reacted with *N,O*-dimethylhydroxylamine hydrochloride (110 mg, 1.12 mmol) in water (acetate buffer, pH 4) for 20 h at room temperature. The solvent was removed, and flash chromatography (water/methanol/chloroform = 0.5:50:50) afforded pure 3e which was finally precipitated in diethyl ether as a white solid (102 mg, 0.27 mmol, 95% yield). $[\alpha]^{25}_D$ = -3.0 (*c* = 0.3, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 2.64 (s, 3H, CH_3N), 3.47 (s, 3H, CH_3O), 3.4-3.8 (m, 11H, H2, H2', H3, H3', H4, H4', H5, H5'and 3 protons among H6 and H6'), 3.87 (dd, 1H, H6 or H6', $^3J_{5-6}$ = 2.1 Hz, $^2J_{6-6'}$ = 12.3 Hz), 4.03 (d, 1H, H1, $^3J_{1-2}$ = 9.0 Hz), 4.34 (d, 1H, H1', $^3J_{1'-2'}$ = 7.8 Hz); ^{13}C NMR (400 MHz, D_2O): δ = 40.4 (C7), 62.4 (C8), 62.6, 63.4 (C6-C6'), 70.9, 71.8, 73.3, 74.8, 77.7, 78.0, 78.6, 80.5 (C2-C2'-C3-C3'-C4-C4'-C5-C5'), 95.0 (C1), 105.2 (C1'); MS: m/z = 386 (M + H$^+$); $C_{14}H_{27}NO_{11}$ (385.4) calcd. C 43.63, H 7.06, N 3.63, found C 43.44, H 7.04, N 3.69.

N,O-Dimethyl-N-(α-D-mannosyl)hydroxylamine. A solution of D-mannose (100 mg, 0.55 mmol) and *N,O*-dimethyl-hydroxylamine hydrochloride (270 mg, 2.2 mmol) in 5 mL of aqueous buffer (sodium acetate/acetic acid 0.1 M, pH 4) was stirred at room temperature. After one week the solvent was evaporated and the product purified by flash chromatography on silica gel (water/methanol/ethyl acetate = 1:24:75) to afford 30 mg of 3f (24% yield). The final product contained 77% of the α-pyranose form and 13% of the α–furanose form that were not isolated but characterised in the mixture. The analysis of the mixture gave the following values:-MS: m/z = 224 (M+H$^+$), 192 (M-OMe); Anal. Calcd. for $C_8H_{17}NO_6$ (223.2): C 43.05, H 7.68, N 6.28; found: C 42.98, H 7.73, N 6.31. *N,O-Dimethyl-N-(α-D-mannopyranosyl)hydroxylamine (3f).* ^{1}H NMR (400 MHz, D_2O): δ = 2.54 (s, 3 H, CH_3N), 3.46 (s, 3H, CH_3O), 3.50 (dd, 1H, H4, $^3J_{3-4}$= $^3J_{4-5}$= 9.0 Hz), 3.62 (dd, 1H, H6, $^3J_{5-6}$= 6.0 Hz, $^2J_{6-6'}$= 12 Hz), 3.70 (m, 1H, H5), 3.74 (dd, 1H, H6'), 3.77 (dd, 1H, H3, $^3J_{2-3}$= 3.4 Hz, $^3J_{3-4}$= 9.0 Hz), 4.05 (d, 1H, H1, $^3J_{1-2}$ = 2.1 Hz), 4.08 (dd, 1H, H2, $^3J_{1-2}$= 2.1 Hz, $^3J_{2-3}$= 3.4 Hz);^{13}C NMR (400 MHz, D_2O): 36.80 (CH_3N), 57.07 (CH_3O), 58.25 (C6), 64.03 (C4), 65.52 (C2), 68.04 (C3), 72.71 (C5), 90.90 (C1). *N,O-Dimethyl-N-(α-D-mannofuranosyl)hydroxylamine.* ^{1}H NMR (400 MHz, D_2O): δ = 2.59 (s, 3 H, CH_3N), 3.48 (s, 3H, CH_3O), 3.5-3.8 (m, 3H, H5, H6, H6'), 3.93 (d, 1H, H4, $^3J_{4-5}$= 9.0 Hz), 4.23 (dd, 1H, H3, $^3J_{3-4}$= 2.5 Hz), 4.33 (dd, 1H, H2, J_{2-3}= 4.3 Hz), 4.47 (d, 1H, H1, $^3J_{1-2}$= 7.0 Hz).

N-Octyl-O-methyl-N-(β-D-glucopyranosyl)hydroxylamine (3g). *N*-Octyl-*O*-methyl hydroxylamine was synthesised from caprylic aldehyde through oxime formation and subsequent reduction with $NaCNBH_3$ as described for compound 2a. A solution of D-glucose (90 mg, 0.5 mmol) and *N*-octyl-*O*-methylhydroxylamine (80mg, 0.5 mmol) in 5 mL of DMF/water (9:1, v/v), was stirred for 20 h at room temperature. The solvent was evaporated and the crude purified by flash chromatography (methanol/ethyl acetate = 1:9) to afford 49 mg of a

colourless oil (30% yield). ^{1}H NMR (400 MHz, D_2O): δ = 0.73 (bt, 3 H, CH_3octyl), 1-1.5 (m, 12H, CH_2octyl), 2.73 (m, 1H, N-CH_A), 2.94 (m, 1H, N-CH_B), 3.21-3.26 (m, 2H, H4 and H5), 3.37 (dd, 1H, H3, $^3J_{2-3}$= $^3J_{3-4}$ = 8.9 Hz), 3.44 (dd, 1H, H2, $^3J_{1-2}$= $^3J_{2-3}$ = 8.9 Hz), 3.48 (s, 3H, OCH_3), 3.58 (dd, 1H, H6, $^3J_{5-6}$= 4.0 Hz, $^2J_{6-6'}$= 11.9 Hz), 3.76 (bd, 1H, H6'), 4.02 (d, 1H, H1, $^3J_{1-2}$ = 8.9 Hz);^{13}C NMR (400 MHz, $CDCl_3$): δ = 16.6 (CH_3octyl), 25.2, 29.9, 30.0, 31.8, 32.0, 34.3 (CH_2octyl), 56.0 (N-CH_2), 63.9 (C6), 65.5 (OCH_3), 71.8 (C3), 72.4 (C4), 79.7 (C2), 80.1 (C5), 95.2 (C1). MS: m/z = 322 (M+ H^+). Anal. Calcd. for $C_{15}H_{31}NO_6$ (321.2): C 56.08, H 9.66, N 4.36. Found: C 56.32, H 9.88, N 4.54.

N-Methyl-hydroxylaminopeptide (5). The tripeptide Ac-Lys(Dde)-Ala-Lys(Boc)-NH_2 was synthesised using Nα-Fmoc chemistry according to the typical procedure for solid phase synthesis. The Nα-Fmoc amino acids side chains were protected as follows: 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) for N-terminal Lys, *tert*-butoxycarbonyl (Boc) for the other Lys. The Nα-Fmoc amino acids were assembled on Rink amide MBHA resin (2g, 0.82 mmol) using the following coupling conditions: Fmoc-amino acid (1.5 equiv.), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (640 mg, 1.5 equiv.) in presence of *N,N*-diisopropylethylamine (DIPEA) (420 µl, 3 equiv.) in 20 mL of dimethylformamide (DMF) for 30 min. Removal of Fmoc protecting groups was achieved by subsequent piperidine cycles (20% v/v in DMF, 3x10 min). N-acetylation of the N-terminal lysine α-amino group was performed with 20 mL of a solution of Ac_2O (10%) and pyridine (10%) in DMF (30 min, room temperature) affording the protected peptide Ac-Lys(Dde)-A-Lys(Boc)-resin. The ε-lysine Dde protecting group was then removed with hydrazine (2%, v/v) in DMF and the succinimide ester of the *N*-Boc,*O*-carboxymethylhydroxylamine (M_r = 288 , 520 mg, 2.2 equiv.) was directly coupled to the peptide-resin in the presence of DIPEA (560 µl, 4 equiv.). The peptide was then cleaved from the resin by treatment with a mixture 2.5% (v/v) of tris-isopropylsilane (TIS), 2.5% water and 85% trifluoroacetic acid (TFA) at room temperature (3 x 1 hour). In these conditions the Boc groups were also cleaved. The combined filtrates were precipitated with diethyl ether, filtered and washed three times with cold diethyl ether. The crude peptide was purified by preparative HPLC (gradient 0% to 50% solvent B in 30 min) to afford 4 as a white powder (R_t= 9.3 min., M_r = 460, 360 mg, 95% yield). ESI-MS: *m/z* = 460.4 (M^+). Peptide 4 (200 mg, 0.43 mmol) was reacted with aqueous formaldehyde (37% solution in water, 50 µl, 1.1 equiv.) in 10 mL of 0.1 M acetate buffer (pH 4.0) and the corresponding *N*-methyloxime-peptide was purified by semipreparative HPLC (gradient of 0% to 50% solvent B in 30 min). After lyophilisation, the pure oxime-peptide was recovered as a white powder (92% yield). The oxime-peptide (M_r = 472, 100 mg, 0.21mmol) was finally reduced with $NaCNBH_3$ (54 mg, 4 equiv.) in 10 mL of acetic acid (10 min, room temperature). The crude peptide 5 was purified by semipreparative HPLC (gradient of 0% to 50% solvent B in 30 min) affording peptide 5 (180 mg, 89%) (R_t= 13.1 min, ESI-MS: *m/z* = 474.7 (M+H).

^{1}H-NMR (400 MHz, D_2O): δ = 1.27 (d, 3H, CH_3 Ala , J = 7.1 Hz), 1.2-1.8 (m, 12 H, $CH_{2\beta}$, $CH_{2\gamma}$, $CH_{2\delta}$ of Lys1 and Lys 3), 1.90 (s, 3H, CH_3CO), 2.72 (bd, 3H, CH_3N), 2.88 (bt, 2H, $CH_{2\varepsilon}$ Lys 3), 3.14 (bt, 2H, $CH_{2\varepsilon}$ Lys 1), 3.22 (s, 2H, CH_2-O-N), 4.05-4.20 (m, 3H, H_α Ala, Lys 1,Lys 3); MS (ESI): m/z = 474.1 (M+H).

General Procedure for Sugar Conjugation to peptide 5 (6a-d). Peptide 5 (20 mg, 0.042 mmol) dissolved in 2 mL of 0.1 M acetate buffer (pH 4) was reacted with the sugar (0.05 mmol). After completion of the reaction and lyophilization, the crude product was purified by preparative HPLC. After 48 h of reaction and HPLC purification, glycopeptide 6a was obtained as a white powder (16 mg, 62%).

^{1}H-NMR (600 MHz, D_2O): δ = 1.26 (d, 3H, CH_3 Ala, J = 7.2 Hz), 1.2-1.8 (m, 12 H, $CH_{2\beta}$, $CH_{2\gamma}$, $CH_{2\delta}$ of Lys 1 and Lys 3), 1.90 (s, 3H, CH_3CO), 2.67 (s, 3H, CH_3N), 2.89 (dd, 2H, $CH_{2\varepsilon}$ Lys 3, $^3J_{\varepsilon-\delta}$ = 7.5 Hz, $^2J_{\varepsilon-\varepsilon'}$ = 4.0 Hz), 3.12 (bt, 2H, $CH_{2\varepsilon}$ Lys 1, $^3J_{\varepsilon-\delta}$ = 8.4), 3.22 (s, 2H, CH_2-O-N), 3.32 (m, 1H, H4 Glc), 3.47 (m, 1H, H5, $^3J_{4-5}$ = 8 Hz Glc), 3.76 (m, 1H, H3 and H2 Glc),3.62 (dd, 1H, H6, $^2J_{6-6'}$ = 12.5 Hz, $^3J_{5-6}$ = 5.9 Hz Glc), 3.80 (dd, 1H, H6', $^2J_{6-6'}$ = 12.5 Hz, $^3J_{5-6'}$ = 1.9 Hz Glc), 4.13 (d, 1H, H1, $^3J_{1-2}$ = 8.7 Hz Glc), 4.15 (m, 1H, H_α Lys 1), 4.21 (m, 1H, H_α Lys 3), 4.25 (m, 1H, H_α Ala 1),MS (ESI): m/z = 635.9 (M+H).

Glycopeptide **6b** was obtained after 144 h at 60 °C (19 mg, 57% yield). R_t 11.1 min (gradient of 0% to 50% solvent B in 30 min). ESI-MS: *m/z* = 798.2 (M+H). Glycopeptide **6c** was obtained after 144 h at 60 °C (14 mg, 35% yield). R_t 10.3 min (gradient of 0% to 50% solvent B in 30 min). ESI-MS: *m/z* = 959.8 (M+H). Glycopeptide **6d** was obtained after 48 h at room temperature (20 mg, 70% yield). R_t 13.6 min (gradient of 0% to 50% solvent B in 60 min), ESI-MS: *m/z* = 677.7 (M+H).

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constants $^3J_{2H-3H}$ and /or $^3J_{3H-4H}$ and/or $^3J_{4H-5H}$ are indicative of a $^4C_1$ conformation. In the case of the D-mannose derivative **3f**, the α form is attributed from a $^1J_{13C1-H1}$ value of about 160Hz[11].

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